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Phenotyping by magnetic resonance imaging nondestructively measures glomerular number and volume distribution in mice with and without nephron reduction

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Reduced nephron mass is strongly linked to susceptibility to chronic renal and cardiovascular diseases. There are currently no tools to identify nephropenia in clinical or preclinical diagnostics. Such new methods could uncover novel mechanisms and therapies for chronic kidney disease (CKD) and reveal how variation among traits can affect renal function and morphology. Here we used cationized ferritin (CF)–enhanced MRI (CFE-MRI) to investigate the relationship between glomerular number (N_{glom}) and volume (V_{glom}) in kidneys of healthy wild-type mice and mice with oligosyndactylism (Os/$^{+}$), a model of congenital nephron reduction. Mice were injected with cationic ferritin and perfused, and the resected kidneys were imaged with 7T MRI to detect CF-labeled glomeruli. CFE-MRI was used to measure the intrarenal distribution of individual glomerular volumes and revealed two major populations of glomeruli distinguished by size. Spatial mapping revealed that the largest glomeruli were located in the juxtamedullary region in both wild-type and Os/$^{+}$ mice and the smallest population located in the cortex. Os/$^{+}$ mice had about a 50% reduction and 35% increase of N_{glom} and V_{glom}, respectively, in both glomerular populations compared with wild type, consistent with glomerular hypertrophy in the Os/$^{+}$ mice. Thus, we provide a foundation for whole-kidney, MRI-based phenotyping of mouse renal glomerular morphology and provide new potential for quantitative human renal diagnostics.

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Nephropenia is strongly associated with susceptibility to chronic kidney disease and cardiovascular disease.[1–4] Although nearly 26 million American adults suffer from chronic kidney disease,[5] there is no effective tool to detect low nephron endowment or nephron loss at the earliest stages. Renal adaptation to low nephron endowment can lead to compensatory glomerular and tubular hypertrophy. The cyclic perpetuation of hyperfiltration and adaption ultimately leads to progressive renal damage, evidenced by an inverse correlation between glomerular number and volume observed postmortem in humans and rodents.[1,6] There are many clinical applications in which knowledge of a patient's nephron number (N_{glom}) and volume (V_{glom}) could have an impact on clinical care or influence medical decision-making—for example, in the evaluation of renal allograft donation, renal assessment following acute kidney injury, or preclinical evaluation of renal toxicity of new therapeutics. The inability to measure N_{glom} and V_{glom} also extends to preclinical models, as there is no tool to monitor nephron number or renal response to reduced nephron number. Such tools could uncover novel mechanisms and therapies for chronic kidney disease and reveal how variation of traits affects renal function and morphology.

Acid maceration and stereology are the predominant methods to assess N_{glom} and V_{glom},[7,8] and they have had a fundamental impact on the understanding of kidney development, health, and disease. However, histological approaches are limited to postmortem analysis after destruction of the kidney. They do not allow for longitudinal analysis, and they do not easily allow three-dimensional (3D) visualization and integration of the renal microstructure.

Preclinical models using transgenic or genetically altered animals are critical to understanding the pathogenesis of renal disease. However, there have been few studies that quantify nephron morphology and variation in rodents. Magnetic resonance imaging (MRI) is gaining interest for renal

phenotyping, because it is noninvasive and it can potentially be performed *in vivo*.[9] Such a tool would be valuable beyond transgenic mouse models to animal models of nephrotoxicity, ischemia, diabetes, and obesity.[10]

There has been increased interest in the development of imaging tools to probe microstructure with MRI.[11,12] Recently, a cationized form of ferritin (the predominant mammalian iron-storage protein) has been developed as a contrast agent for MRI to measure kidney glomerular morphology.[10,13–17] Ferritin forms an ~13-nm nanoparticle with a metal oxide core. Cationic ferritin (CF) is functionalized with amines,[18] and it has an isoelectric point of up to ~9.[13] After intravenous injection, CF nanoparticles bind to anionic proteoglycans in the glomerular basement membrane (GBM). The iron oxide core of the CF nanoparticles is detected by T^*_2—shortening in MRI that produces a dark punctate artifact at the site of CF accumulation.[13] CF-enhanced MRI (CFE-MRI) has enabled measurements of N_{glom} and V_{glom} in intact isolated rat kidneys *ex vivo*[14,15] and in isolated human donor kidneys.[19] CFE-MRI has also been used to image nephrons *in vivo* in rats.[13,17,20]

Here we used CFE-MRI to generate 3D maps of perfused glomeruli and measure N_{glom} and V_{glom} in healthy mouse kidneys *ex vivo*. We further applied CFE-MRI to investigate the oligosyndactylism (Os/$^+$) mouse model of congenital nephron reduction, to determine how N_{glom} and V_{glom} are affected by reduced renal mass. The Os/$^+$ mouse has a mutation on chromosome 8 secondary to radiation exposure with two dominant phenotypic manifestations including (1) fusion of the second and third digits on each limb and (2) reduced nephron number.[21–25] Homozygous inheritance results in death shortly after implantation.[26–29] The Os/$^+$ mutation disrupts *Anapc10*, which encodes for component 10 of the anaphase-promoting complex/cyclosome (APC/C). Although there is a human ortholog to this gene (*ANAPC10*), which resides on chromosome 10, there is no prior report of a mutation in vertebrates.[30]

We compared MRI-based measurements with histological estimates and investigated the intra- and interrenal distribution of individual glomerular volume (IGV). This work provides the foundation for whole-kidney, MRI-based phenotyping of mouse renal glomerular morphology.

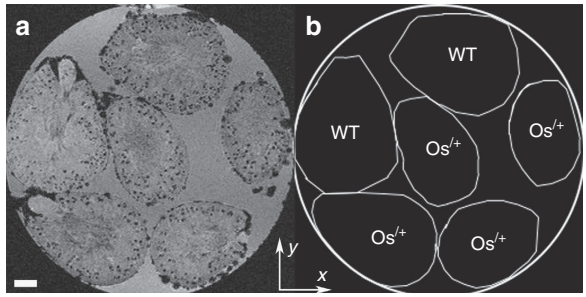

Figure 1 | Kidneys labeled with cationized ferritin (CF) were imaged together in a single three-dimensional magnetic resonance image scan and separated in postprocessing for analysis. Axial image (**a**) with its corresponding identification maps (**b**) shows clear CF labeling in all kidneys at 7T. The axial plane is defined orthogonal to the main B_0 magnetic field. Scale bar = 1 mm. Os/$^+$, oligosyndactylism; WT, wild type.

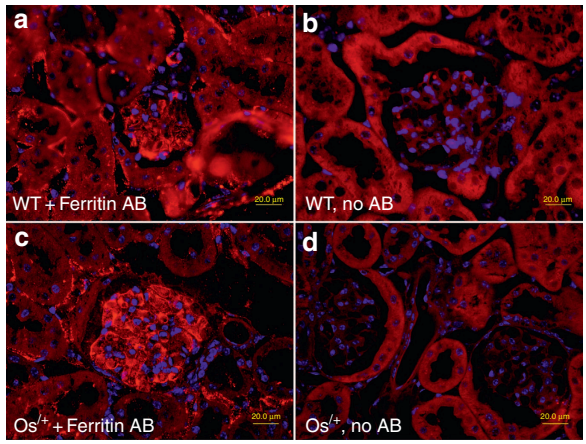

Figure 2 | Detection of cationized ferritin (CF) labeling in healthy wild-type (WT) and oligosyndactylism (Os/$^+$) mouse kidneys after retro-orbital injection using immunofluorescence imaging. Sections were stained with anti-horse spleen ferritin (AHSF) (**a** and **c**) and without AHSF (**b** and **d**). Both (**a**) and (**c**) images show fluorescence in the glomerular basement membrane (GBM), indicating CF uptake in WT and Os/$^+$ glomeruli. Sections that were not stained with the AHSF (**b** and **d**) showed no GBM labeling.

RESULTS

We imaged 10 CF-labeled (5 WT, 5 Os/$^+$) and two unlabeled mouse kidneys with 7T MRI using a 3D gradient recalled echo pulse sequence. MR images (Figure 1a) of CF-labeled kidneys exhibited dark spots in the cortex, consistent with the accumulation of CF in individual glomeruli.[13,14,19] Images of the kidneys from unlabeled mice exhibited no spots.[13,14] Binding of CF to the GBM was confirmed with immunofluorescence in WT and Os/$^+$ mice, as shown in Figure 2.

We segmented each kidney from original 3D MR images. Our custom software identified CF-labeled glomeruli from the 3D MR images and measured the number of labeled glomeruli in each kidney. We segmented glomeruli and cortex in optical images and used histological methods to estimate N_{glom} and V_{glom} (Figure 3). MRI-based measurements yielded an average N_{glom} of $12{,}010 \pm 447$ (WT) and 5632 ± 1279 (Os/$^+$). Stereological estimates yielded average N_{glom} of $11{,}660 \pm 1091$ (WT) and 5561 ± 1954 (Os/$^+$). Acid maceration estimates of average N_{glom} were $10{,}364 \pm 1123$ (WT) and 4281 ± 655 (Os/$^+$). Unlabeled kidneys imaged with MRI gave an average count of 1075. The average Dice coefficient for WT and Os/$^+$ kidneys was 0.945, indicating that MRI-based detection was robust. Original MR images of kidneys in a multikidney holder and corresponding identification maps are shown in Figure 1.

The perturbation caused by CF-labeled glomeruli in MRI extended several voxels, as seen in Figure 4a. We calculated

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